REDUCING PROTEIN A LEACHING DURING PROTEIN A AFFINITY CHROMATOGRAPHY

This application is a continuation under 37 C.F.R. §1.53(b) of U.S. patent application Ser. No. 10/877,532 filed Jun. 24, 2004, now U.S. Pat. No. 7,485,704, which is a non-provisional application claiming priority under 35 U.S.C. §119 to U.S. Provisional Patent Application Ser. No. 60/490,500 filed Jul. 28, 2003, the entire disclosures of which are hereby incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention concerns protein purification. In particular, the invention concerns a method for reducing leaching of protein A during protein A affinity chromatography by reducing temperature or pH of, or by adding one or more protease inhibitors to, a composition that is subjected to protein A affinity chromatography.

2. Description of Related Art

The large-scale, economic purification of proteins is increasingly an important problem for the biotechnology industry. Generally, proteins are produced by cell culture, using either mammalian or bacterial cell lines engineered to produce the protein of interest by insertion of a recombinant plasmid containing the gene for that protein. Since the cell lines used are living organisms, they must be fed with a complex growth medium, containing sugars, amino acids, and growth factors, usually supplied from preparations of animal serum. Separation of the desired protein from the mixture of compounds fed to the cells and from the by-products of the cells themselves to a purity sufficient for use as a human therapeutic poses a formidable challenge.

Procedures for purification of proteins from cell debris initially depend on the site of expression of the protein. Some proteins can be caused to be secreted directly from the cell into the surrounding growth media; others are made intracellularly. For the latter proteins, the first step of a purification process involves lysis of the cell, which can be done by a variety of methods, including mechanical shear, osmotic shock, or enzymatic treatments. Such disruption releases the entire contents of the cell into the homogenate, and in addition produces subcellular fragments that are difficult to remove due to their small size. These are generally removed by differential centrifugation or by filtration. The same problem arises, although on a smaller scale, with directly secreted proteins due to the natural death of cells and release of intracellular host cell proteins in the course of the protein production run.

Once a clarified solution containing the protein of interest has been obtained, its separation from the other proteins produced by the cell is usually attempted using a combination of different chromatography techniques. These techniques separate mixtures of proteins on the basis of their charge, degree of hydrophobicity, or size. Several different chromatography resins are available for each of these techniques, allowing accurate tailoring of the purification scheme to the particular protein involved. The essence of each of these separation methods is that proteins can be caused either to move at different rates down a long column, achieving a physical separation that increases as they pass further down the column, or to adhere selectively to the separation medium, being then differentially eluted by different solvents. In some cases, the desired protein is separated from impurities when the impurities specifically adhere to the column, and the protein of interest does not, that is, the protein of interest is present in the "flow-through."

Affinity chromatography, which exploits a specific interaction between the protein to be purified and an immobilized capture agent, may also be an option for some proteins. Protein A is a useful adsorbent for affinity chromatography of proteins, such as antibodies, which contain an Fc region. Protein A is a 41kD cell wall protein from *Staphylococcus aureas* which binds with a high affinity (about 10^{-8}M to human IgG) to the Fc region of antibodies.

U.S. Pat. Nos. 6,127,526 and 6,333,398 (Blank, G.) describe an intermediate wash step during protein A affinity chromatography using hydrophobic electrolytes, e.g., tetramethylammonium chloride (TMAC) and tetraethylammonium chloride (TEAC), to remove the impurities, but not the immobilized protein A or the protein of interest, bound to the protein A column.

SUMMARY OF THE INVENTION

The present invention concerns a method of purifying a protein which comprises a C_H2/C_H3 region, comprising reducing the temperature of a composition comprising the protein and one or more impurities subjected to protein A affinity chromatography in the range from about 3° C. to about 20° C., wherein protein A leaching is reduced.

Preferably the protein is an antibody, e.g. one which binds an antigen selected from the group consisting of HER2, vascular endothelial growth factor (VEGF), IgE, CD20, CD40, CD11a, tissue factor (TF), prostate stem cell antigen (PSCA), interleukin-8 (IL-8), epidermal growth factor receptor (EGFR), HER3, HER4, $\alpha4\beta7$ or $\alpha5\beta3$. In another embodiment, the protein is an immunoadhesin, such as a TNF receptor immunoadhesin.

The invention also concerns a method of purifying a protein which comprises a C_H2/C_H3 region by protein A affinity chromatography comprising:

(a) subjecting the protein to protein A affinity chromatography and measuring leached protein A in a composition comprising the protein which is recovered from the protein A affinity chromatography;

(b) if protein A leaching is detected in step (a), reducing the temperature of a composition comprising the protein and one or more impurities subjected to protein A affinity chromatography in the range from about 3° C. to about 20° C., such that protein A leaching is reduced.

The invention further provides a method for reducing leaching of protein A during protein A affinity chromatography comprising reducing protease activity in a composition subjected to protein A affinity chromatography, wherein the composition comprises a protein which comprises a C_H2/C_H3 region and one or more proteases.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** depicts protein A leaching as a function of temperature for various antibody products on PROSEP A™. Leached protein A is shown in ng/mg (ng protein A per mg antibody). Temperature on the x-axis refers to the temperature of the water bath. The column was equilibrated and washed with 25 mM Tris, 25 mM NaCl, 5 mM EDTA, pH 7.1, washed with 25 mM Tris, 25 mM NaCl, 0.5 M TMAC, 5 mM EDTA pH 5.0 or 7.1, eluted with either 25 mM citrate pH 2.8, or 0.1 M acetic acid pH 2.9, regenerated with 0.1 M phosphoric acid, and stored in 0.2 M sodium acetate, 2% benzyl alcohol pH 5.0. Trastuzumab was run on a bed height of 20 cm, loaded to 20